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Chemoreceptive Control of Feeding Processes in Hydra

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Abstract

Cnidarians are the simplest metazoans to exhibit satiety after feeding. When hydra are fed to repletion, they close their mouths and cease to capture prey. As feeding stops, contractions of the tentacles and body column increase. Our earlier experiments showed that a gel chromatographic fraction of prey substances inhibits prey capture. We now present evidence that the same fraction reduces the duration of mouth opening induced by reduced glutathione (GSH) and inhibits the binding of GSH to its putative receptor. The fraction also induces column contractions which are similar to those normally seen in sated animals. Prey substances, of unfractionated homogenate, also induce post-feeding tentacle contractions similar to those seen in sated animals. Gut distention does not appear to induce behavior associated with satiety. Therefore, these experiments suggest that chemoreception of prey substances induce satiety in hydra. **Chem. Senses 21: 313–321, 1996.**

Introduction

Feeding behavior in hydra has been well documented. It consists of a series of sequential behavior patterns, including prey capture, mouth opening, ingestion, digestion and regurgitation. Mechanical and chemical stimuli, produced by swimming prey, induce the hydra to discharge its nematocysts (Ewer, 1947; Lubbock, 1979; Watson and Hessinger, 1989; Thorington and Hessinger, 1990; Brinkmann and Thurm, 1991; for review see Kass-Simon and Hufnagel, 1992). Once these pierce the prey, reduced glutathione (GSH), leaking from the prey, causes the hydra's tentacles to writhe and its mouth to open (Loomis, 1955; Lenhoff, 1961). The writhing tentacles bring the prey to the hydra's mouth, which opens to ingest it (Lenhoff, 1974). After ingestion, endodermal cells, lining hydra's gut, digest the prey and undigested material is then regurgitated (Lenhoff, 1969).

If hydra are fed to repletion, they cease to capture prey, even though nematocysts in the tentacles are not depleted (Burnett *et al.*, 1960; Smith *et al.*, 1974; Ruch and Cook, 1984; Grosvenor and Kass-Simon, 1987). Our earlier study showed that a water soluble fraction (FR1), isolated from disrupted prey by gel chromatography, inhibits prey capture when applied to the solution surrounding the hydra; presumably by association with a putative ectodermal receptor (Grosvenor and Kass-Simon, 1987).

In order to look at the effects of FR1 on the entire feeding behavior, we performed a series of experiments in which we:

(i) electrophysiologically monitored the behavior of hydra during feeding and the early post-feeding phases
(ii) measured the duration of mouth opening in the presence of GSH and in the presence of prey fractions
(iii) measured the binding of GSH to its putative receptor in the presence of the FR1.

We find that FR1 reduces the duration of mouth opening in the presence of GSH, inhibits the binding of GSH to its putative receptor and induces body contractions similar to those observed during post-feeding behavior. We also show that unfractionated homogenate induces periods of intense tentacle contractions which are behavioraly distinct from the effects of FR1 or GSH. Therefore, just as reduced glutathione and other substances turn on the feeding behavior of coelenterates (Loomis, 1955; Lenhoff, 1961; Fulton, 1963a, b; Mariscal and Lenhoff, 1968; Lindsted, 1971; Reimer, 1971; Lehman and Porter, 1973), it now appears that prey substances can also switch it off and turn post-feeding behavior on.

Materials and methods

All reagents were purchased from Sigma Chemical Co. (St Louis, MO) with the exception of [^{35}S]-glutathione, which was purchased from New England Nuclear (Boston, MA, 80–114 Ci/mmol). Reduced glutathione (GSH) solutions were prepared in culture solution. Whole homogenate (10.1 + 0.69 mg protein/ml) was prepared from *Artemia nauplii* that were disrupted by trituration in culture solution. Following centrifugation (1000 × *g* for 10 min), 1.5 ml aliquotes of the H_2O soluble layer were stored at −15°C. Aliquotes were thawed and recentrifuged (1000 × *g*) for 10 min prior to use (Grosvenor and Kass-Simon, 1987). Homogenate was fractionated in a Sephadex G-25–80 column (1.5 × 17 cm). FR1 was the first fraction from the column with a relative mobility (M_r) >1500 Da. Removal of GSH from FR1 was verified by the addition of 5 nM of [^{35}S]-radiolabeled-GSH to whole homogenate prior to fractionation. A total of 99.8% GSH was removed from FR1 as determined by liquid scintillation spectrometry. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951), using bovine albumin as the standard.

Hydra vulgaris (attenuata) were maintained in modified BVT culture solution (Loomis and Lenhoff, 1955), containing 1.2×10^{-3} M $NaHCO_3$, 2.16×10^{-4} M $CaCL_2$ and 1.7×10^{-4} M EDTA in distilled water. They were fed an excess of *Artemia nauplii* alternate days and starved 2 days prior to experimentation, unless otherwise stated. Hydra can be starved up to 4 days or more and remain healthy (Fraser and Bode, 1981).

To determine the effects of FR1 on the GSH-induced mouth-opening response, we placed six hydra in 60 × 15-mm petri dishes, containing BVT culture solution. The initial culture solution was replaced by culture solution containing various concentrations of GSH, with or without FR1 (1/100 dilution), or containing 5 μM GSH with various FR1 dilutions. We recorded the time from GSH addition to the initiation of mouth opening and the total duration of the response—from the addition of GSH to mouth closure. Subtracting the time required for the mouth to open from the total duration, gives the duration of the mouth opening response.

Hydra membrane preparation and binding methods are given in Bellis *et al.* (1991) and Grosvenor *et al.* (1992). A crude membrane fraction was prepared by homogenizing 500 two-day starved hydra over ice. A 30 000 × *g* pellet was recovered from the homogenate using differential centrifugation in which a 1000 × *g* pellet was discarded. The 30 000 × *g* pellet was resuspended in 50 mM Tris-HCl and was incubated (75–100 μg protein/ml) at 25°C for 25 min, with 20 nM [^{35}S]-GSH (100 Ci/mmol) to measure total binding and with 20 nM [^{35}S]-GSH plus 1000-fold excess unlabeled GSH to measure non-specific binding. Incubations were terminated by filtration (MSI cellulose filters, 0.45 μm). Filters were rapidly washed twice with ice-cold tris buffer (4 ml) and radioactivity was measured using scintillation spectrometry. All assays were run in duplicate. Specific binding is operationally defined as the difference between determinations of total and non-specific binding. Specific binding was also determined in the presence of varying dilutions of FR1.

To look at the behavioral physiology of feeding in hydra, we made electrophysiological recordings during and after feeding, in the presence of whole prey homogenate and in the presence of FR1. In order to obtain an electrical and behavioral description of the normal post-feeding behavior in hydra, 1-day starved animals were attached to polyethylene suction electrodes in culture solution and recorded from while being observed through a dissecting microscope. Signals were amplified and recorded on a Model 79 Grass Polygraph (EEG preamplifier). Ten minutes after electrode attachment, the hydra were fed an excess of *Artemia nauplii* (20–30) until they stopped feeding. Initiation of post-feeding behavior was defined by the cessation of prey capture and ingestion. Recording and observation continued for 90 min after ingestion ceased. The number of individual tentacle

pulses and column contraction bursts, which were recorded and correlated to behavior, were counted. Contraction bursts were defined as two or more contraction pulses occurring within 30 s of each other which were accompanied by column contractions. Preliminary experiments indicated that there were no significant differences in the number of pulses per burst under the different experimental conditions. For analysis, the records were aligned to make the time-points at which feeding ceases coincide. Records were compared statistically from 30 min prior to 90 min after the point of feeding cessation.

To test the effects of prey substances upon tentacle pulses and contraction bursts, hydra were both perfused and injected with test solutions (whole homogenate or FR1), with test solution and culture solution, or with culture solution alone. Whole homogenate was the water soluble fraction of disrupted *Artemia nauplii* after centrifugation at 1000 × *g* for 15 min (Grosvenor and Kass-Simon, 1987). Perfusions were made through an injection port at the base of a 15-ml recording chamber; 1-µl injections of undiluted samples were made through the body wall near the basal pore of the hydra with a Hamilton syringe bearing a drawn-out polyethylene tip. These injections caused the distention and filling of the gut. Samples for perfusion were diluted 1/50 (a concentration that gives a pronounced response). At this dilution, the samples had a mean protein concentrations of 200 and 27 µg/ml for homogenate and FR1, respectively. Tentacle pulses and contraction bursts were counted only if they were both recorded on the chart recorder and visually observed as indicated by an event mark on the chart recorder.

In an additional set of experiments, the reversibility of the effects of perfused FR1 was measured. FR1 samples were diluted 1/100 (13.5 µg protein/ml) and perfused over the hydra without injection. Hydra were first perfused with culture solution for 15 min. They were then perfused with diluted FR1, followed by two culture solution rinses. As in the previous experiments, the hydra were adapted to the electrode for 10 min prior to initiating the experiments.

Statistical analyses were done on a PC using Systat software (Evenston, IL). Where applicable, Wilcoxon Rank Sums, Kruskal–Wallis Tests and Friedman Two-Way Analyses were done. Follow-up tests were performed manually (Hollander and Wolfe, 1973).

Results

The duration of mouth opening was measured in the presence of GSH alone and in the presence of GSH plus FR1 (Figure 1A). The duration of mouth opening was dependent upon the GSH concentration. Maximal duration was observed at a concentration of 5 µM GSH, with a 50% response (EC_{50}) at approximately 1 µM GSH concentrations. The addition of 1/100 dilutions of FR1 (mean protein concentration equal to 14.5 µg/ml) caused a significant inhibition of the mouth

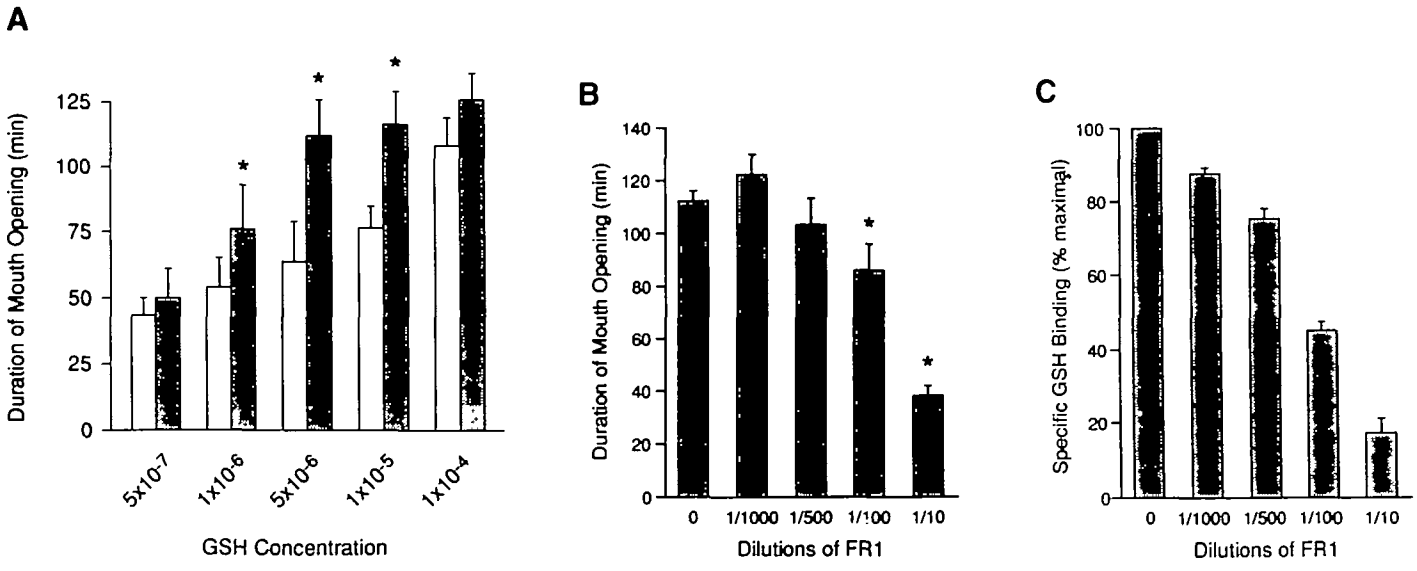

Figure 1 **(A)** Mouth opening in increasing GSH concentrations (grey bars); together with 1/100 dilution (14.5 µg/ml protein) of FR1 (white bars). Each bar represents the mean ± SE for six determinations of six animals each. The * indicates the pairs of values which are significantly different from each other. **(B)** Mouth opening in 5 µM GSH, together with increasing concentrations of FR1: mean ± SE for six determinations of six animals each. The * indicates the dilutions of FR1 which are significantly different from the sample in which no FR1 is added. **(C)** Percentage maximal specific GSH binding to membrane preparations in the presence of varying dilutions of FR1. Mean ± SE four determinations performed in duplicate. All samples were significantly different from each other.

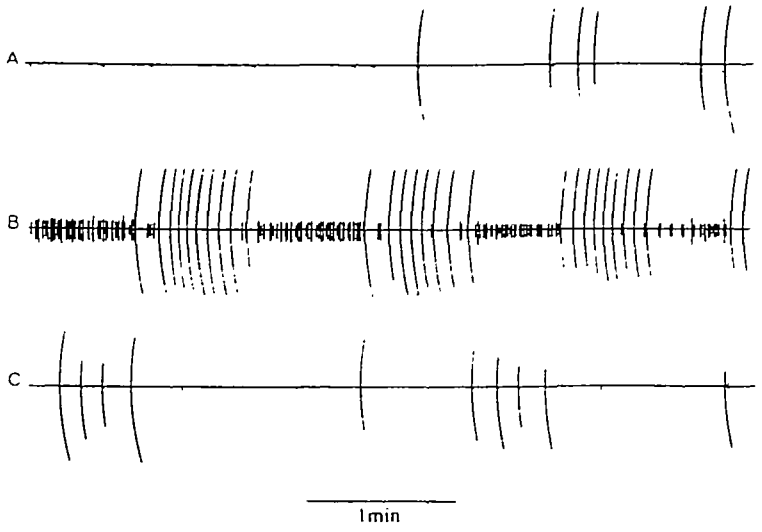

Figure 2 Five-minute samples were taken from a continuous record of hydra during 30 min of feeding (to satiation = cessation of prey capture) followed by 90 min of post-feeding behavior. (1) Twenty minutes after food introduction (during feeding), (2) after 40 min (early post-feeding); (3) after 100 min (late post-feeding). Large pulses are visually correlated with body contractions Small pulses are associated with tentacle contractions

opening response at higher GSH concentrations. At a GSH concentration of 5 μM, FR1 significantly reduced the duration of the mouth opening response by 41% of that of animals untreated with FR1 ($P < 0.05$, Wilcoxon Rank Sums). At 100 μM, inhibition was no longer significant ($P > 0.05$), nor did FR1 have an effect at the lowest GSH concentration ($P > 0.05$).

In further experiments, the duration of mouth opening was measured at 5 μM GSH plus varying dilutions of FR1. Mouth opening decreased progressively as the concentration of FR1 was increased (Figure 1B). At a 1/1000 dilution (1.4 μg protein/ml), there was no effect; at 1/100 dilution (13.8 μg/ml), there was a 32% decrease; at 1/10 dilution (138 μg/ml), the decrease was 67%. Since the solutions were externally applied, this reduction in mouth opening could not be due to the filling of the gut.

These results could be explained if FR1 inhibited the binding of GSH to putative receptor sites. We measured the specific binding of [^{35}S]-GSH to a membrane fraction of hydra, either in the presence or absence of FR1. Increasing concentrations of FR1 progressively inhibited GSH binding to the putative receptor sites (Figure 1C). At an FR1 dilution of 1/100 (20.8 μg protein/ml), binding was 55% inhibited; at a 1/10 dilution (208 μg/ml), binding was 83% inhibited. Therefore, prey substances comprising FR1 inhibited GSH binding to its putative ectodermal receptor site in the same concentration range at which they decrease the duration of GSH-induced mouth opening.

We hypothesized that these or additional prey substances

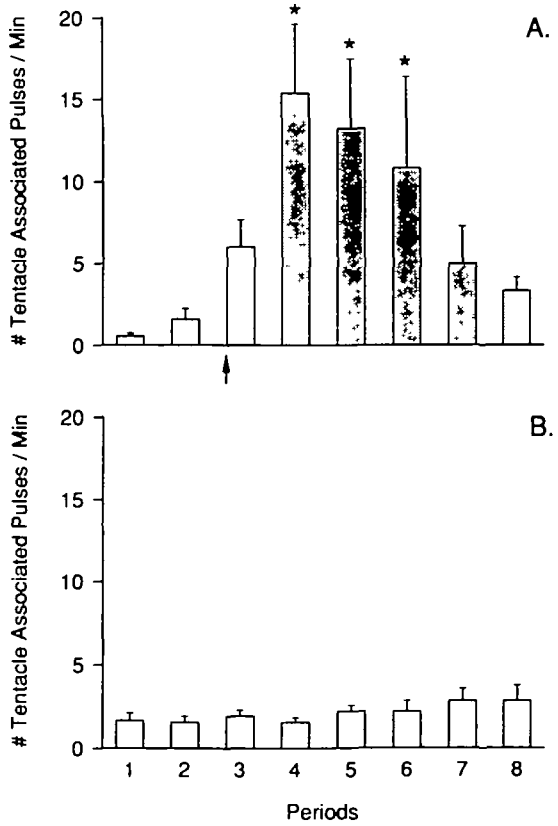

Figure 3 Histogram of the number of tentacle pulses, from the polygraph records (including that shown in Figure 2), during the feeding and post-feeding phases. Tentacle pulses/min were calculated for each 15-min interval of continuous 2 h records. The records are aligned so that the points where feeding ceased coincide. In **(A)** the arrow indicates the start of the post-feeding phase. **(B)** Unfed hydra were recorded from for 2 h (control). Mean ± SE for six determinations of six animals each. Significant differences between corresponding periods of fed and unfed hydra are indicated by *'s.

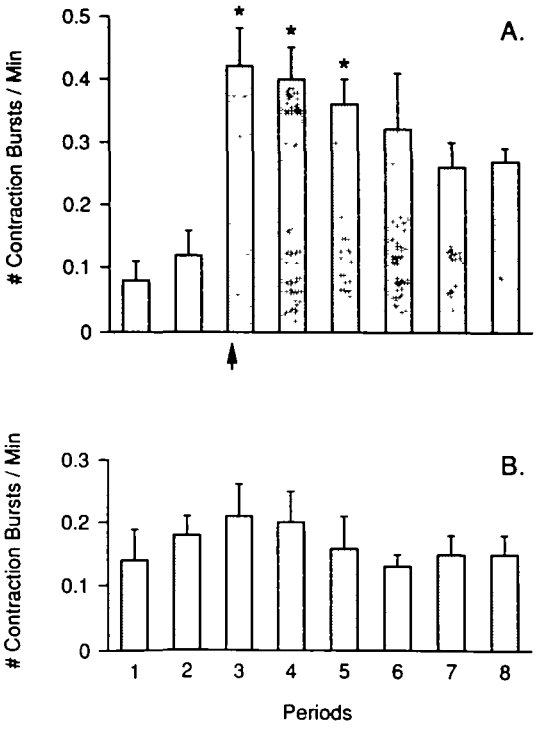

Figure 4 Histogram of the number of contraction bursts during the feeding and post-feeding phases in hydra, from records described in Figure 2. The records from six animals were alligned as in Figure 3. Contraction bursts/min were calculated for each 15-min interval of continuous 2 h records. In **(A)** the arrow indicates the start of the post-feeding phase. **(B)** Unfed hydra were recorded from for 2 h (control). Mean ± SE (n = 6). Significant differences (P > <0.05) between corresponding periods of fed and unfed hydra are indicated by *'s.

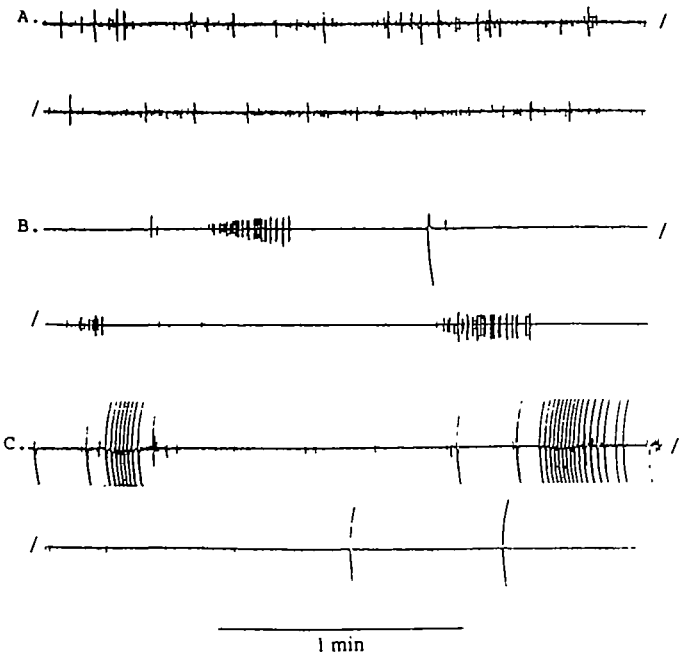

Figure 5 Representative records from whole animal recordings of hydra indicating increases in tentacle pulses or contraction bursts after application of whole homogenate or FR1, respectively. The records are the middle 5 min of a 15-min recording after beginning of the treatment. **(A)** A high gain recording of a hydra perfused and injected with culture solution (controls). **(B)** An animal was injected and perfused with whole homogenate, showing an increase in bursts of tentacle pulses. **(C)** An animal, injected and perfused with FR1, had an increase in contraction bursts, but not tentacle pulses.

present in FR1 might elicit post-feeding behaviors normally seen in satiated animals. The effect of prey substances on post-feeding behavior was measured by recording from animals with extracellular suction electrodes. Marked changes occurred in the pattern of tentacle pulses and column contraction bursts during feeding and post-feeding periods (Figure 2). When hydra were feeding, there were few tentacle pulses or contraction bursts (Figure 2A). After prey capture ceases, tentacle pulses and contraction bursts increase in frequency. Figure 2B shows the increase in the number of both tentacle pulses (small pulses) and contraction bursts (groups of large pulses) during the early post-feeding phase. These decreased in frequency during the course of digestion (Figure 2C). The increase in tentacle pulses (Figure 3A) and contraction bursts (Figure 4A) was significant ($P < 0.05$, Wilcoxon Rank Sums) compared to the same time periods for unfed control animals (Figures 3B and 4B).

We next looked into the question of the effects of prey substances on the behavioral physiology of feeding. Our electrophysiological records indicated that tentacle activity, associated with post-feeding behavior, was not the same as that produced by GSH (Rushforth and Burke, 1971; Rushforth and Hofman, 1972). GSH causes slow tentacle writhing which is accompanied by electrical waves. Whereas, post-feeding tentacle contractions are accompanied by distinct high frequency impulses. In order to determine whether the increases in tentacle pulses and contraction bursts were due to substances from the prey or distension of the gut, and to determine where putative receptors may be located, hydra were simultaneously perfused and injected with whole homogenate, FR1 or culture solution. Perfusion of whole homogenate with simultaneous injection of culture solution, produced significant increases (×92) in the number of tentacle pulses compared to control animals, in which culture solution was both perfused and injected ($P < 0.05$, Kruskal–Wallis, Figure 6). Tentacle pulses were also significantly increased (×32) when whole homogenate was injected with simultaneous perfusion of culture solution. Perfusion and simultaneous injection of FR1 also significantly increased the number of tentacle pulses by ×62. However, examination

of the responses of individual animals showed that the statistical increase was due to tentacle pulse stimulation in only two of six animals. The other four animals, in which tentacle pulses were not increased, were not different from the controls.

Perfusion and simultaneous injection of whole homogenate induced a ×522 increase in the number of tentacle pulses, which was significantly larger than all other samples ($P < 0.05$, Kruskal–Wallis, Figures 5B and 6). Distension of the gut alone does not induce tentacle pulses, since neither perfused FR1 plus injected culture solution nor injected FR1 with perfused culture solution, had an effect. In addition, injection of 200 mOsm sorbitol, having a similar osmolarity to whole homogenate, with simultaneous perfusion of dilute sorbitol (1/50) did not induce tentacle pulses, indicating that the high osmolarity of the homogenate was not the stimulating agent.

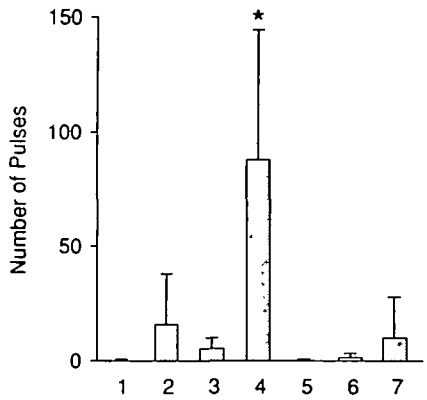

Figure 6 Whole homogenate injected and perfused caused a significant increase in the number of tentacle pulses compared to all other treatments. The hydra were subjected to the following treatments: (1) injected and perfused with culture solution (controls), (2) perfused with whole homogenate; (3) injected with whole homogenate, (4) perfused and injected with whole homogenate; (5) perfused with FR1; (6) injected with FR1, (7) perfused and injected with FR1. Mean ± SE of six determinations of six animals each. The * indicates the sample which was significantly different from all others.

When FR1 was simultaneously perfused and injected, the number of contraction bursts increased significantly (×5.5, Figures 5C and 7A, $P < 0.05$, Kruskal–Wallis) compared to untreated controls. Similar increases in contraction bursts (×5 compared to controls) were found when FR1 was perfused with culture solution injection. In addition, animals treated with whole homogenate (perfused and/or injected) and animals injected with FR1 (perfused with culture solution) did not differ from controls ($P > 0.05$, Kruskal–Wallis). Since perfused and perfused/injected FR1 have the same effect, external application alone is sufficient to produce an increase in contraction bursts. Injection of either homogenate or FR1 (perfusion of culture solution) also significantly increased the number of contraction bursts compared to control animals (×2.6 and ×2.3, respectively). As with the induction of tentacle pulses, a change in the osmolarity of the medium did not cause an increase in the number of contraction bursts, since diluted FR1 used in the perfusate has the same osmolarity as culture solution (3 mOsM). In another set of experiments in which FR1 was perfused (no injection) over the hydra and then washed out with culture solution (Figure 7B), the removal of FR1 from the medium

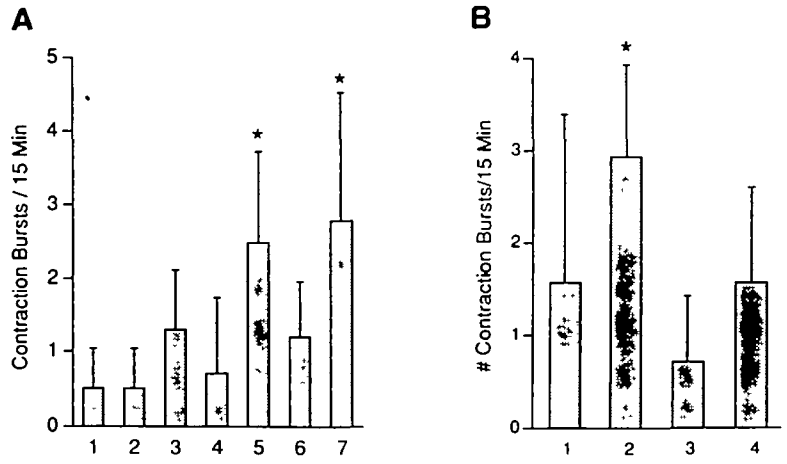

Figure 7 **(A)** Histogram showing that fraction 1 perfused over hydra induces contraction bursts. The hydra were subjected to the following treatments (1) injected and perfused with culture solution (controls); (2) perfused with whole homogenate; (3) injected with whole homogenate; (4) perfused and injected with whole homogenate; (5) perfused with FR1; (6) injected with FR1, (7) perfused and injected with FR1. Mean ± SE, $n = 6$, for each treatment. The * indicate those treatments that are significantly different from the control values. **(B)** Histogram indicates that the effects of FR1 are reversible. The hydra were initially perfused with culture solution (1), followed by FR1 (2) and then two-culture solution washes (3,4). Mean ± SD, $n = 14$ The * indicates the sample which is significantly different from all other samples.

rapidly reduced the number of contraction bursts to control levels, indicating that the effects of FR1 were reversible.

Discussion

Our previous study demonstrated that application of whole homogenate to the culture solution surrounding a hydra caused mouth opening, tentacle writhing and the complete inhibition of prey capture. A crude separation of the homogenate with gel chromatography yielded a fraction (M_r > 1500 Da) which did not cause mouth opening or tentacle writhing, but inhibited prey capture, even though the tentacles were not contracted (Grosvenor and Kass-Simon, 1987). The present study indicates that this same fraction can inhibit mouth opening and induce column contractions in a way similar to that observed in fully fed hydra and that prey substances can also induce tentacle contractions similar to those observed in fed animals.

Using Lenhoff's (1961) behavioral assay, we found that the duration of the mouth opening response was longest at a concentration of 5 μM GSH and half maximal at 0.5–1 μM GSH. These values are comparable to those obtained by Lenhoff with *H. littoralis*, although the maximum duration of the response is longer in *H. vulgaris*.

A 1/100 dilution of FR1 (13.8–20.8 μg protein/ml) significantly reduces the duration of mouth opening in the presence of varying concentrations of GSH (Figure 1A). The inhibitory effect of FR1 was eliminated at a higher GSH concentration. This is consistent with the notion that GSH and FR1 are competitors as suggested by the direct binding studies with [^{35}S]-GSH. However, a model of simple competitive inhibition must be ruled out, since FR1 does not affect the feeding response at low concentrations. If the interaction was simple competition the effect of FR1 should have been maximal at low GSH concentration.

Hanai *et al.* (1987), studying *H. japonica*, has presented evidence to suggest that hydra may have multiple components to their feeding response. They found that several blood platelet proteins had differential effects upon the tentacle ball formation response. Each protein specifically depressed the ball formation at different concentrations of S-methylglutathione (a GSH agonist), which they suggest may indicate five subsets of the GSH receptor. If there were more than one GSH receptor subset, FR1 may only inhibit those receptor subsets that are induced at higher concentrations of GSH. Increasing the concentration of FR1 caused a progressive decrease in the duration of mouth opening (Figure 1B). This correlated with the radioligand binding data in that increasing FR1 concentration caused an increasing inhibition of GSH association to its receptor site.

As shown in Figure 2, when hydra become satiated, they produce post-feeding behavior which involves both tentacle and column contractions. These appear to be mediated by chemoreceptive recognition of prey substances by hydra. Column contractions can be induced by FR1. A 1/50 dilution of FR1 (Figure 7A) caused a 5-fold increase in the number of electrically recorded contraction bursts, which is similar to that observed in fully fed animals (Figure 2B). As with the effects of FR1 on the mouth opening response, external application alone was sufficient to elicit a response. Unlike other invertebrates (Gelperin, 1972; Kuslansky *et al.*, 1987), gut distension is not required to evoke behavioral satiety in hydra.

Although either perfusion or injection of homogenate increases the number of tentacle pulses, by far the largest increase was caused by both internal and external presence of prey substances. This would suggest that both internal and external receptors are involved in causing the response. This situation may be similar to neck formation in hydra, which requires the presence of tyrosine in the gut and GSH in the external medium for the response to be expressed (Blanquet and Lenhoff, 1963). The induction of tentacle or column contractions cannot be attributed to alterations in osmolarity, since diluted FR1 has the same osmolarity as culture solution and replacing whole homogenate with isosmotic sorbitol, under the same experimental conditions, does not induce tentacle pulses.

Both termination of mouth opening and the activation of post-feeding behavior appear to be induced by the interaction of prey substances with putative receptors of the hydra. The termination of mouth opening may be due to prey substances which inhibit the binding of GSH to its external receptor site, thus inactivating the mouth opening response to GSH. Receptor sites that may be involved with the activation of tentacle pulses are likely to be located both on the internal and external surface of the hydra, since whole homogenate must be present both in the gut and on the external surface of the hydra for maximal induction of the response. Although injected FR1 partially induced contraction bursts, perfused FR1 caused a greater stimulation. This implies that receptors for FR1 are principally located on the external surface.

The electrical impulses that induce column contractions may arise from either the hypostome (Passano and McCullough, 1964) or the base of a hydra (Kass-Simon, 1973). It has also been shown that electrical impulses, which initiate contractions of tentacles, may also induce

contractions of the whole animal (Kass-Simon 1972, 1973; Rushforth 1973). During the early post-feeding phase (Figure 2B), the consistent appearance of tentacle pulses prior to contraction bursts suggested that tentacle pulses may initiate—hence drive—contraction bursts. However, during the later post-feeding phase (Figure 2C), contraction bursts can occur without any associated tentacle pulses. We show that contraction bursts can be induced by perfused FR1, which does not initiate tentacle pulses. Perfused and injected whole homogenate, which induces tentacle pulses, does not cause an increase in contraction bursts. Therefore, as suggested by Rushforth (1971, 1972) and others, tentacle pulse and contraction burst pacemaker systems can be completely uncoupled and may, in fact, be independently stimulated by different substances or combinations of substances. Further fractionation of prey substances would lead to a better understanding of the active components in hydra prey, their interactions with the putative GSH receptor subtypes and the regulation of tentacle pulses and contraction bursts.

In summary, when hydra are fully fed, inhibition of prey capture and termination of mouth opening appear to occur almost simultaneously. Hydra will no longer capture any prey brushing the tentacles. Prey already captured will not be ingested and will eventually fall to the bottom of the dish. At the same time, the number of column contractions intensifies to an apparent maximal intensity. When the hydra contract, substances can sometimes be seen leaking from the mouth. Our experiments indicate that these behavior patterns, which represent operationally defined satiety in hydra, may be due to the binding of prey derived substances to external and internal receptors to induce the behaviors associated with satiety.

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